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An innovative co-composting approach for fish-farm sapropel and cattle manure: Humification dynamics and initial agrochemical properties

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ABSTRACT

Overuse of mineral fertilizers leads to the degradation of soils and decreases their humus content; thus, alternative sources need to be considered. The goal of this paper was to create an organomineral fertilizer based on co-composting of fish-farm sapropel and cattle manure and analyze the humification process, physicochemical properties, and biological activities of the created compost. The sapropel collected from the Syrdarya region was mixed with cattle manure using different mass proportions (90:10–10:90) and was kept aerobically for 120 days, with moisture content being controlled (30–50%). It was established that the duration of the composting process and the ratio of the substrate have a significant impact on the humification of the compost and its mineral composition. The ideal ratio gave the highest level of maturity (humification percentage is equal to 44.12%, gaseous losses are only 1.72%) of the compost. On the basis of the data from the microbiological study, it can be concluded that active microbial flora is present. In the course of bioassay tests, extracts of compost significantly positively influenced the germination of cotton seeds and their development. It can be said that co-composting of sapropel with cattle manure is a promising and effective way of obtaining organic fertilizer.

Keywords: sapropel, manure, composting, organic fertilizer, humification extent, number of microorganisms, sapropel extract, cotton seeds.

INTRODUCTION

Global population growth is projected to continue, with the United Nations estimating a rise to ~9.7 billion by 2050. This demographic expansion coincides with a persistent decline in per-capita arable land and increasing pressure on freshwater resources, thereby intensifying constraints on global food security and the long-term sustainability of agricultural systems. The analyses linking food demand to land and water availability indicate that, between 1960 and 2050, global food requirements are expected to increase while the availability of key natural resources per person continues to contract, reinforcing the urgency for resource-efficient production pathways (Ibarrola and Sander, 2016). For example, the estimates reported in the literature show a marked decline in cropland availability per capita—from approximately 0.22 ha per person (1975) to 0.12 ha (2002)—with projections suggesting further reductions to ~0.08 ha per person by 2050, reflecting growing land scarcity at the household level.

Although chemical fertilizers are effective in maintaining crop productivity, they unfortunately pose a risk to the preservation of humus, the key component of soil fertility. Long-term evidence indicates that continuous application of mineral fertilizers at the full recommended rate can reduce the soil humus content by approximately 0.11 percentage points within three years, whereas the use of organic fertilizers tends to increase humus accumulation by replenishing soil organic matter and stimulating humification processes (Kloster *et al.,* 2024; Lal 2015; Guo *et al.,* 2010). The presence of humus in the soil is one of the key indicators of soil fertility. To restore the humus balance, organic fertilizers are traditionally applied, including manure, poultry litter, peat, oxidized coal, green manures such as legumes, cereals, cruciferous and amaranth plants, as well as chopped straw, plant residues, leaves, and other biomass sources (Shamuratov *et al.,* 2023). The application of organic fertilizers improves three fundamental soil properties: agrochemical, agrophysical, and biological (Liu *et al.,* 2024; Wang and Zhang, 2023). In addition, the use of organic fertilizers supports the maintenance of a positive humus balance in the soil, increasing its content by 0.06 to 0.14 percent, depending on the application rate (Kloster *et al.,* 2024; Lal 2015; Liu *et al*., 2024; Wang and Zhang, 2023).

In recent years, growing attention has focused on sapropel—a nutrient-rich organic sediment formed in freshwater bodies—because of its potential as a biologically active soil amendment and fertilizer (Zarina *et al.,* 2023). Sapropel is a pasty material typically containing 70–75% moisture. It is generally odorless and tasteless and is characterized by high plasticity and viscosity, as well as a strong adsorptive capacity. Its color varies depending on the environmental conditions under which it formed. Freshwater lakes may contain up to three types of sapropel, with composition varying by depth, distance from the shoreline, and prevailing environmental conditions. Sapropel contains up to 75 percent organic matter, essential nutrients, such as nitrogen, phosphorus, and calcium oxide, trace elements, amino acids, and vitamins, including vitamin A. Its application improves soil structure, enhances fertility, and stimulates biological activity (Yuldasheva *et al.,* 2026). The studies conducted at the Priekulsky Centre have shown that sapropel application can improve crop yield and product quality, including higher protein content in beans and increased starch accumulation in potatoes For light grey forest soils, the optimal application rate is 30 tons per hectare, particularly when combined with the Azofoska mineral fertilizer. Sapropel contributes to improved plant growth and enhanced soil fertility (Vasiliev *et al*., 2022). The organic matter content of sapropel varies with its quality grade and classification; however, some sources indicate that it should be no less than 15%. In contrast, the national standard GOST R 54000-2010 “Organic fertilizers. Sapropels. General specifications” specifies that first-class sapropel must contain ≥50% organic matter on a dry-matter basis (GOST R 54000-2010). To improve this characteristic, sapropel is commonly co-composted with manure, peat, and other organic residues. Co-composting promotes organic matter stabilization and nutrient transformation, thereby enhancing the agrochemical and agrophysical properties of the material and increasing its effectiveness as a soil amendment and fertilizer (Janušienė, 2003; Kopytkov, 2022; Sylvia, 2020).

The composts prepared using sapropel in combination with inorganic materials such as phosphogypsum and bentonite, along with organic residues including manure, poultry litter, straw, husks, and peat, have proven effective in enhancing the properties of sapropel. Experimental results showed that the maize plants grown with these composts exhibited increased biomass and height, confirming the potential of sludge-based composts for improving crop productivity (Mamas and Lebedov, 2014; Nagorny and Lyashko, 2012; Kurakov and Bilanenko, 2023; Saber, 2023; Blečić *et al.,* 2014; Obuka, 2021; Sotimboev *et al.,* 2024).

The composting of various organic wastes in Azerbaijan, including livestock manure, municipal solid waste, poultry droppings, and sapropel, has been studied with decomposition periods ranging from four to twelve months. The resulting compost contains approximately 4.8% nitrogen, 1.0% phosphorus, 1.5% potassium, and up to 60% organic matter, underscoring its value as a source of plant nutrients and organic inputs for improving soil properties. Field applications across multiple regions have demonstrated consistent benefits for crop productivity. In the Zagatala region, maize yields increased to 10 t ha^{-1}; in Absheron, grape yields rose to 15 kg ha^{-1}, accompanied by a 3.6% increase in sugar content; in Lankaran, green tea production increased by 250–300 kg ha^{-1}; and in Neftchala, cotton yields increased by 3.2 kg ha^{-1} (Zamanova, 2019). This study aimed to evaluate the production of organic fertilizer (OF) via aerobic composting of the sapropel collected from fish farms in the Syrdarya region of Uzbekistan, combined with cattle manure, under room-temperature incubation. Optimizing humification under low carbon-to-nitrogen (C/N) conditions can be effectively achieved by adjusting the sapropel-to-cattle manure mixing ratio and varying the incubation period. This strategy improves the humification process and, consequently, enhances cotton seed germination energy and viability. These outcomes are further supported by microbiological characterization of both the initial feedstocks and the resulting compost. Regression and variance analyses, including ANOVA with significance levels of $P < 0.05$ and $P < 0.01$, as well as Tukey's post hoc test, were employed to evaluate the experimental results. These analyses enabled the identification of the optimal sapropel-to-manure mass ratio and its effects on compost quality, germination energy, and seed viability in cotton (Sultan-Elita variety).

MATERIALS AND METHODS

Composting materials and analytical methods for composition determination

The sapropel used in this study was collected in February 2024 from the shoreline of a lake located at the Gayrat Fayzli Dalasi fish farm in the Syrdarya region of Uzbekistan. Sampling was conducted under ambient conditions: air temperature of 12 °C, relative humidity of 55 percent, and atmospheric pressure of 632 mm Hg.

The second material used to produce OF was cattle manure (CM). The moisture content in sapropel, manure, and compost samples was determined gravimetrically by GOST 26712–94, using a Binder ED 115 drying oven at 100–105 °C until a constant weight was achieved. The mass fraction of organic matter was analyzed following the protocols outlined in GOST 27980–80 and GOST R 54000–2010. The pH value was measured electrometrically in a 10% aqueous suspension using a Mettler Toledo FiveGo ion meter (Switzerland).

Humic acids (HA) were extracted using a 0.1 N sodium hydroxide (NaOH) solution at a temperature not exceeding 80 °C over 24 hours. The resulting alkaline extract was acidified to pH 2 with concentrated hydrochloric acid, leading to the precipitation of HA, which was subsequently separated by filtration (Dragunov 1957). The content of HA was determined gravimetrically based on the mass of the precipitate. The fulvic acid (FA) content in the compost was determined by calculating the difference between the total amount of alkali-soluble organic matter and HA content (Tingyu *et al.,* 2023). The content of water-soluble organic matter (WOM) was determined by heating a sapropel sample in a water bath at a temperature not exceeding 80°C for 2 hours. All analyses for HA, FA, and WOM were conducted in triplicate. Each procedure was performed in parallel on two identical samples to ensure reproducibility and accuracy. The difference between alkali-soluble and alkali-insoluble organic matter represents the insoluble residual organic matter, also referred to as non-hydrolyzable organic residue (NOR). To determine this fraction, the sediment was thoroughly washed with distilled water and dried to a constant weight, enabling calculation of the residual organic mass. This value was used to quantify the content of residual organic matter in the sample. Total nitrogen was determined using the Kjeldahl method (De Caro, Cosimo, 2004). The ash content of the samples was determined according to GOST 11306–83 by incinerating the material in a CNOL muffle furnace (Russia) at 800 °C until a constant mass of residue was achieved (Adeeva *et al.,* 2009). The total organic matter (TOM) content was calculated as the difference between the mass of the dry sample and the mass of the resulting ash. The organic carbon content was determined by wet combustion using a mixture of potassium dichromate, sulfuric acid, and phosphoric acid (Methods, 2009). To assess the chemical and elemental composition, the sapropel and manure samples were first dried at 100 °C and then calcined at 800 °C before being digested in aqua regia (a 1:3 mixture of concentrated nitric acid [HNO_3] and hydrochloric acid [HCl]). Elemental analysis was performed using a Shimadzu ICPE-9000 spectrometer (Japan) equipped with inductively coupled argon plasma, operating over a wavelength range of λ = 179–769 nm. The results of the physical, chemical, and elemental composition of sapropel are presented in Table 1.

The primary quality indicators for fertilizers include the standardized content of heavy metals, such as cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg). It was established that the concentrations of these elements in sapropel ash were

Table 1. Comparative physicochemical and elemental characteristics of sapropel and cattle manure

Characteristics	Results	
	Sapropel	Manure
pH	7.54	7.25
Moisture content %	39.45	19.01
Dry matter%	60.55	80.99
In dry matter:		
Moisture %	2.01	-
Total nitrogen, %	0.36	0.96
Organic matter. %	9.49	54.24
Content of humic acids. %	4.85	6.20
Content of fulvic acids. %	3.74	6.42
Content of water-soluble organic matter. %	0.33	6.06
Content of non-hydrolyzable organic residue. %	0.57	18.68
Content of ash. %	54.75	26.75
Content of macroelements:		
SiO_2	55.06	-
$SiO_{2soluble}$	0.37	-
CaO	13.05	0.94
MgO	2.09	0.13
P_2O_5	0.17	0,43
$FeO+Fe_2O_3$	4.30	0.28
TiO_2	0.45	-
Na_2O	1.11	0.79
K_2O	0.97	1.39
SO_3	<0.16	-
$C_{organic}$	0.78	-
Loss on ignition	12.54	-
Content of microelements in ash, mg/kg:		
Pb	15.24	-
Cu	19.76	58.1
Zn	62.27	2584
As	14.76	-
Hg	7.619	-
Cd	0.106	-
Mn	311.02	35699

as follows (mg/kg): Cd – 0.106, As – 14.762, Pb – 15.240, and Hg – 7.619. These values do not exceed the permissible limits established by GOST R 54000–2010. In addition, the presence of 0.37% soluble SiO_2 in the raw material should be noted, as it represents an important parameter that enhances the potential agronomic value of the resulting fertilizer.

Composting process

The composting process was investigated using various mass ratios of sapropel (SP) and cattle manure (CM), ranging from 90:10 to 10:90. Composting was conducted under facultative aerobic conditions at room temperature (25 °C) over an incubation period of 120 days. Considering the high moisture-retention capacity of sapropel, additional water was added to each mixture to maintain optimal moisture content in the range of 30–50%, which is essential for effective humification of organic matter.

The prepared mixtures were placed in 0.5 L plastic containers, and a thin layer of soil was applied to the surface of each container. The containers were incubated in a thermostat at a constant temperature of 25 °C. The samples for chemical composition analysis were collected at 30-day intervals throughout the composting period. For comparison, the composting processes of the individual raw materials—sapropel and manure—were also studied under identical conditions.

Instrumental analyses

The microstructural images of raw and ash sapropel were obtained using a scanning electron microscope equipped with energy-dispersive X-ray spectroscopy (SEM–EDX), model JSM-6610LV (JEOL, Japan). For scanning electron microscopy (SEM) analysis, the sapropel sample was mounted onto a conductive carbon adhesive tape affixed to a removable aluminum alloy stage. The study was conducted using a JSM-6610LV microscope (JEOL, Japan). Surface morphology was examined using secondary electron imaging at an accelerating voltage of 20 kV.

Microbial analysis

The total number of heterotrophic microorganisms in sapropel, cattle manure, and compost samples after 150 days of composting was determined using the limiting dilution method (Yamamoto *et al.,* 2009).

Method of determination

Under strict aseptic conditions, a series of tenfold dilutions of the test sample suspension (from 10^1 to 10^6) was prepared using sterile distilled water. An aliquot of each diluted suspension (e.g., 1 mL) was transferred into sterile Petri dishes, followed by the addition of melted and cooled (45–48 °C) nutrient-rich agar medium (meat-peptone agar [MPA] or glucose-yeast agar [GRA]). The contents of the dishes were gently mixed and incubated at 28–30 °C for 24–72 hours, depending on the sample type. After incubation, the number of bacterial colonies on each plate was counted and recalculated per gram of sample. The results were expressed as colony-forming units (CFU) per gram. All bacteriological analyses were performed in triplicate.

Assessment of germination rate and germination energy of cotton (*Gossypium* spp.) seeds

In a laboratory experiment, the effect of sapropel extracts at concentrations ranging from 0.1% to 0.001% on the germination rate and energy of cotton seeds was evaluated by GOST 12038–84 "Seeds of Agricultural Crops. Methods for Determining Germination". Germination energy was defined as the percentage of the seeds that germinated within a short period, specifically at 3 and 6 days for cotton.

Each crop has specific timeframes for assessing germination energy and viability, as outlined in GOST 12038–84 "Seeds of Agricultural Plants". These timeframes vary significantly depending on the crop species. Germination energy serves as a key indicator of seed quality, as it reflects the ability of seeds to produce uniform and timely seedlings, often a critical factor in achieving high agricultural yields. The seeds with low germination energy are generally less tolerant to biotic and abiotic stressors, whereas the seeds with high germination energy demonstrate greater competitiveness against weeds, pathogens, and unfavorable environmental conditions.

Germination energy is defined as the uniformity of seed germination and shoot emergence and is expressed as the percentage of seeds that germinate within a specified period (GOST 12038–84). This parameter can also be used to study the effects of various substances (e.g., their aqueous solutions) on seed germination, providing a basis for approximating their potential impact on subsequent stages of plant ontogenesis (Frayssinet *et al.,* 2019).

For a preliminary assessment of agrochemical efficiency, the effect of aqueous sapropel suspensions on the germination energy and germination rate of cotton seeds was investigated. During the experiment, moisture-retaining materials (e.g., filter paper) were placed in Petri dishes for seed placement. Cotton seeds (10 per dish) were presoaked for 20 hours in the respective test solutions before placement.

Over one week, tests were conducted to assess germination energy and seed viability. By following the procedure for primary phytotesting outlined in GOST 12038–84 "Seeds of Agricultural Crops. Methods for Determining Germination", both germination percentage and germination energy were evaluated during the first week. On the sixth day of incubation, additional measurements were taken to determine the average shoot length and fresh biomass (raw weight) of the seedlings.

The experiment was conducted according to the following design:

- Control – seeds were soaked in distilled water;
- Experimental treatments – seeds were soaked in aqueous extracts of ground sapropel at concentrations of 0.1% (10 g/100 mL H_2O), 0.01%, and 0.001%. The lower concentrations were calculated based on the rate of organic matter application to the soil.

Seed germination was carried out in Petri dishes lined with filter paper, placed in a thermostat at 25 °C in the dark. The experiment was repeated three times to ensure reproducibility.

Measurements and calculations

The degree of humification of organic matter in the finished composts (C_{hum}) was calculated using the following formula:

$$C_{hum} = \frac{(G_{HA} + G_{FA} + G_{WOM})}{G_{TOM}} \cdot 100 \quad (1)$$

where: G_{TOM} – total content of organic matter in the compost (g); G_{HA} – content of humic acids (g); G_{FA} – the content of fulvic acids (g); G_{WOM} – content of water-soluble organic matter (g).

The loss of organic matter (*L*OM) into the gas phase during composting was calculated using the following formula:

$$L_{loss} = 1 - \frac{(G_n - G_{n+15})}{2a} \cdot 100 \quad (2)$$

where: G_n — initial mass of organic matter in the compost mixture (g), G_{n+15} — the total organic matter content (g) measured every 15 days, where *n* denotes the number of days since the start of composting

Statistical analysis

The experimental data were processed using the Statistica 10.0 software (StatSoft, USA). The reliability of differences between mean values was evaluated using Student's *t*-test. To assess the effects of two independent factors and their interaction on the measured parameters, a two-way analysis of variance (ANOVA) was performed with calculation of the corresponding F-statistics. Statistical significance was determined at confidence levels of 95% ($p < 0.05$) and 99% ($p < 0.01$). The results are presented as arithmetic means with standard deviations (M ± SD), along with confidence intervals at $p < 0.05$.

RESULTS AND DISCUSSION

Microstructural analysis of the original dried sapropel and its ash revealed that the raw sapropel predominantly contains particles of irregular shape (Figure 1a). In contrast, cubic-shaped particles were observed in the ash fraction (Figure 1b).

Scanning electron microscopy (SEM) analysis of the surface morphology of raw sapropel (Figure 1a) revealed the presence of mineral particles with varying degrees of dispersion, closely associated and agglomerated with organic matter, indicating strong structural integration between the organic and inorganic components. Clay and quartz particles ranging from 5 to 15 µm in size exhibited irregular edges and rough surfaces. Other particles displayed non-uniform or elongated morphologies, with sizes ranging from 1 to 5 µm. Additionally, the SEM images showed the presence of voids with widths ranging from 25–50 µm, and in some areas exceeding 100 µm (Figure 1a).

Morphological analysis of sapropel ash (Figure 1b) revealed the presence of compacted particle aggregates with pronounced structural heterogeneity. The ash composition includes discrete, rounded formations approximately 20 µm in diameter, as well as elongated, strip-like structures measuring 2–5 µm in thickness and 100–150 µm in length. Furthermore, compaction of the ash matrix resulted in a noticeable reduction in pore size, predominantly within the range of 10–25 µm.

Table 2 presents the results of composting experiments using mixtures of sapropel and manure in varying proportions. The data show that as the mass fraction of sapropel decreases and the proportion of manure increases, there is a corresponding rise in the contents of carbon, nitrogen, total organic matter, humic and fulvic acids, water-soluble organic matter, and the carbon-to-nitrogen (C/N) ratio.

Additionally, an increase in incubation time beyond 30 days led to a gradual decrease in total organic matter, accompanied by an increase in the concentrations of humic acids, fulvic acids, and water-soluble organic matter.

The relationship between the humification degree and the loss of composting products into the gas phase were investigated. The corresponding results are presented in Figures 2a and 2b.

As the data indicate, increasing the incubation time promotes a higher degree of humification. In contrast, increasing the proportion of manure relative to sapropel leads to a reduction in the humification degree. For instance, at SP:CM = 90:10, the humification degree rises progressively from 34.38% after 1 day to 40.21%

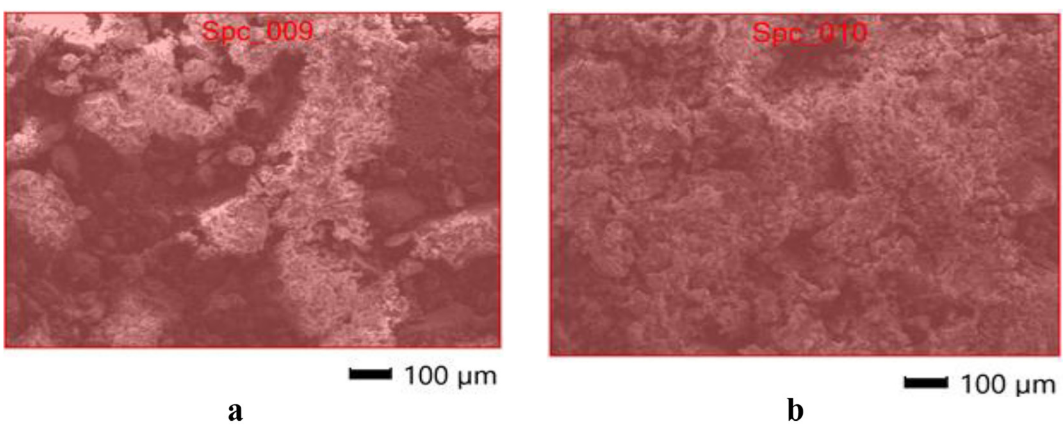

Figure 1. SEM images of (a) dried sapropel and (b) sapropel ash, showing differences in particle morphology and porosity

Table 2. Temporal dynamics of organic and nutrient composition in composts derived from the Syrdarya fish farm sapropel and cattle manure, as influenced by incubation duration and substrate mixing ratios

Mass ratio SP:CM	$W_{hum\text{-}idit,}$ %	pH of 10% solution	C, %	N, %	TOM, %	HA, %	FA, %	WOM, %	C/N
On day 30									
SP	33.00	7.50	0.73	0.25	8.65	1.27	1.31	1.24	2.95
CM	51.08	7.20	7.13	1.08	53.62	6.41	6.63	6.26	6.60
90:10	31.09	7.20	1.50	0.32	15.57	2.08	2.15	2.03	4.69
70:30	43.10	7.27	3.47	0.48	23.47	2.98	3.08	2.91	7.23
50:50	46.08	7.30	4.51	0.66	31.6	3.9	4.03	3.81	6.83
30:70	54.07	7.38	5.15	0.83	40.47	4.91	5.08	4.79	6.20
10:90	55.85	7.39	5.75	1.00	44.85	5.43	5.62	5.30	5.75
Std. Dev.	9.82	0.11	2.30	0.33	16.27	1.86	1.92	1.81	1.49
CI <0.05	10.31	0.12	2.41	0.34	17.07	1.95	2.02	1.90	1.56
CI <0.01	16.17	0.18	3.78	0.54	26.78	3.06	3.17	2.99	2.45
10:90	55.84	7.37	5.82	1.02	44.65	5.55	5.74	5.42	5.71
Std. Dev.	9.82	0.12	2.31	0.33	16.26	1.86	1.92	1.82	1.41
CI <0.05	10.31	0.12	2.43	0.35	17.07	1.95	2.02	1.91	1.48
CI <0.01	16.17	0.19	3.81	0.55	26.77	3.06	3.17	2.99	2.32
On day 60									
SP	33.00	7.48	0.74	0.25	8.27	1.49	1.54	1.45	2.88
CM	51.03	7.17	7.20	1.13	53.21	6.63	6.86	6.47	6.37
90:10	31.02	7.17	1.56	0.34	15.18	2.31	2.38	2.24	4.59
70:30	43.04	7.22	3.53	0.52	23.08	3.20	3.31	3.12	6.79
50:50	46.02	7.27	4.57	0.69	31.21	4.11	4.24	4.00	6.62
30:70	54.01	7.35	5.23	0.87	40.07	5.10	5.28	4.98	6.01
10:90	55.80	7.36	5.84	1.04	44.45	5.65	5.85	5.52	5.61
Std. Dev.	9.82	0.11	2.32	0.34	16.26	1.85	1.92	1.81	1.39
CI <0.05	10.30	0.12	2.43	0.36	17.06	1.95	2.02	1.90	1.46
CI <0.01	16.16	0.19	3.81	0.56	26.76	3.05	3.16	2.99	2.29
On day 90									
SP	35.00	7.43	0.74	0.26	8.00	1.61	1.67	1.57	2.84
CM	50.50	7.12	7.22	1.15	52.93	6.74	6.97	6.58	6.28
90:10	30.85	7.18	1.56	0.36	14.91	2.41	2.49	2.35	4.33
70:30	42.95	7.23	3.55	0.53	22.79	3.31	3.42	3.23	6.70
50:50	45.90	7.27	4.59	0.73	30.92	4.21	4.36	4.11	6.29
30:70	53.88	7.35	5.25	0.89	39.77	5.21	5.39	5.08	5.90
10:90	55.56	7.33	5.86	1.06	44.15	5.81	6.01	5.67	5.53
Std. Dev.	9.36	26.53	2.33	0.34	16.25	1.86	1.92	1.82	1.37
CI <0.05	9.82	27.84	2.44	0.36	17.05	1.95	2.02	1.91	1.44
CI <0.01	15.41	43.67	3.83	0.56	26.75	3.06	3.17	2.99	2.25
On day 120									
SP	34.45	7.40	0.75	0.28	7.91	1.63	1.69	1.59	2.67
CM	50.01	7.09	7.24	1.16	52.85	6.76	6.99	6.60	6.23
90:10	30.15	7.14	1.61	0.37	14.83	2.43	2.51	2.37	4.35
70:30	42.26	7.18	3.61	0.56	22.71	3.33	3.44	3.25	6.45
50:50	45.32	7.24	4.63	0.74	30.83	4.23	4.37	4.13	6.26
30:70	53.41	7.30	5.32	0.89	39.69	5.23	5.41	5.11	5.98
10:90	55.16	7.27	5.91	1.08	44.19	5.83	6.03	5.69	5.47
Std. Dev.	19.31	0.10	2.33	0.34	16.27	1.86	1.92	1.82	1.38
CI <0.05	20.27	0.11	2.44	0.36	17.07	1.95	2.02	1.91	1.45
CI <0.01	31.79	0.17	3.83	0.56	26.78	3.06	3.17	2.99	2.27

Note: *CI <0.05 – Student confidence interval P <0.05, CI <0.01 – Student confidence interval P <0.01.

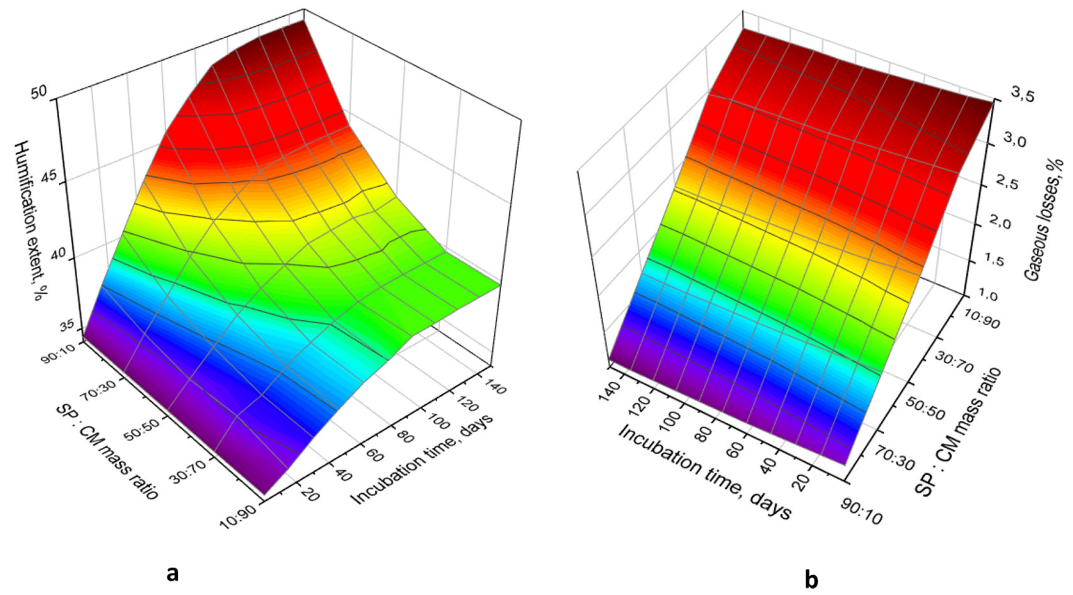

Figure 2. Dependence of humification degree (a) and gaseous losses (b) on incubation time and SP : CM mass ratios

(30 days), 45.58% (60 days), 48.62% (90 days), and 49.29% (120 days). A similar trend is observed across other SP:CM ratios, with higher sapropel content consistently resulting in more advanced humification over time.

However, this trend is inversely related to the loss of substances into the gas phase. Specifically, an increase in incubation time leads to a reduction in gaseous losses. At the same time, a higher proportion of manure in the sapropel-to-manure mixture results in a greater release of volatile substances. This phenomenon may be attributed to the lower content of mineral components in sapropel, which contributes to structural stability, and the higher concentration of readily degradable organic matter in manure, which enhances microbial activity and gas emissions during composting.

For example, at SP:CM ratio of 90:10, the loss of volatile substances into the gas phase decreased significantly from 1.24% to 1.11% as the composting period increased from 1 to 120 days. In contrast, reducing the sapropel content leads to a shift in the SP:CM ratio from 90:10 to 10:90, resulting in a notable increase in gaseous losses, rising from 34.38% to 34.45% and from 38.51% to 39.72%, respectively, over the same incubation period. These findings confirm that higher manure content enhances the release of volatile compounds during composting, while prolonged incubation reduces such losses over time.

The results of the statistical analysis enabled the identification of the most favorable conditions for producing OF. Among the tested treatments, the SP : CM mass ratio of 70:30 combined with a composting duration of 90 days was found to be optimal in terms of nutrient composition, degree of humification, and minimal gaseous losses. Under these conditions, OF exhibited the following characteristics (wt.%): TOM – 22.79%; HA – 3.31%; FA – 3.42%; WOM – 3.23%; H_2O – 42.95%; pH – 7.23. The degree of humification reached 43.70%, while the loss of organic substances into the gas phase was limited to 1.74%, indicating an efficient and stable composting process. Two-way ANOVA confirmed that both the SP:CM ratio ($F = 48.3$, $p < 0.01$) and incubation duration ($F = 31.7$, $p < 0.01$) had statistically significant independent effects on the degree of humification, while their interaction was also significant ($F = 6.9$, $p < 0.05$), indicating that the optimal ratio depends on composting duration. Tukey's HSD post hoc test revealed that the SP:CM = 70:30 treatment at 90 days was significantly superior to all other ratios ($p < 0.05$), confirming it as the statistically optimal combination for maximizing humification, while minimizing gaseous losses.

In the samples of sapropel and cattle manure before composting, as well as in composts after 120 days of incubation, the total number of heterotrophic microorganisms was determined using the limiting dilution method. Enumeration was

Table 3. Descriptive statistics of microbial populations detected on organic and mineral nitrogen media in initial feedstocks and mature compost (SP:CM = 70:30)

Sample	Number of colonies on			
	MPA(×10⁵ CFU/g)		KAA (×10⁵ CFU/g)	
	Average	SD	Average	SD
Sapropel	1.06	0.16	4.02	0.41
Cattle manure	7.61	0.83	44.08	2.79
Compost	7.20	0.35	10.01	0.42
MS	779.956		1400.911	
p-value	0.0017		0.0000000	
F	6.502246		515.5844	
F крит	2.9466		5.143253	

performed on two nutrient media: meat-peptone agar (MPA) to assess the microorganisms utilizing organic nitrogen sources, and SAA to quantify those capable of assimilating mineral nitrogen sources (Table 3).

Before incubation, the sapropel sample contained 1.06 × 10^5 CFU/g on meat-peptone agar (MPA), of which approximately 5% were actinomycetes, with the remainder consisting predominantly of bacteria. On starch-ammonia agar (SAA), the total count was 4.0 × 10^5 CFU/g, with actinomycetes comprising around 4%. In contrast, the cattle manure sample exhibited a higher microbial load: 7.6 × 10^5 CFU/g on MPA, consisting entirely of spore-forming microorganisms, notably Bacillus mycoides; and 4.4 × 10^6 CFU/g on SAA, primarily bacteria with 10% actinomycetes. Following 120 days of incubation of the sapropel-manure mixture, the microbial community underwent substantial transformation. While the total CFU count decreased by approximately 5.3%, the physiological and morphological diversity of the colonies indicated the establishment of a distinct and functionally specialized microbial consortium, characteristic of the matured compost (Bustamante *et al.,* 2010).

On the surface of the rich meat-peptone agar (MPA), abundant growth of morphologically diverse microbial colonies was observed, exhibiting variations in shape, size, margin characteristics, pigmentation, texture, and transparency. The pronounced heterogeneity of colony morphology suggests the presence of a mixed microbial community, comprising bacterial taxa, such as *Bacillus*, *Pseudomonas*, and *Micrococcus*, as well as possibly yeast-like organisms, identified by their smooth, convex, and glossy colony appearance. This complex and diverse microbial profile is characteristic of saprophytic aerobic microflora, commonly associated with mature and biologically active composts.

On SAA, the abundance of microorganisms capable of assimilating mineral nitrogen was significantly higher than in sapropel, but remained lower than in the original compost. The presence of colonies with greater morphological diversity on SAA is indicative of compost maturity and enhanced biological activity, which is typical of the organic substrates that have undergone advanced decomposition (Meng et al., 2019). The colony morphology observed on SAA was consistent with that of actinomycetes, predominantly of the genus *Streptomyces*, which are commonly associated with soil–compost microbiota. The presence of these microorganisms reflects a high level of cellulolytic activity, confirming the biological maturity of compost. Furthermore, their occurrence suggests the potential antiphytopathogenic properties, as *Streptomyces* spp. are well known for their ability to synthesize antibiotic compounds. These findings are consistent with the recent international studies on sapropel-based composts: Blečić et al. (2014) reported similar actinomycete proliferation in sapropel-manure co-composts, while Sylvia et al. (2020) documented comparable CFU dynamics during aerobic composting of freshwater sapropel. In contrast to mineral nitrogen-based fertilizers, which tend to suppress soil microbial diversity (Liu et al., 2024), the compost produced in this study demonstrated enriched microbial community structure, further supporting its value as a biologically active organic amendment.

Descriptive statistics of microbial counts on nutrient media, along with the results of multiple comparisons using Tukey's honest significant

Table 4. Results of multiple comparisons (according to Tukey HSD)

Comparison among samples	Difference in means	p-value	Significance
Comparison by MPA (CFU × 10^5):			
Compost of cattle manure	0.41	0.633	No
Compost of sapropel	6.14	<0.001	High
Compost of SP : CM = 70 : 30	6.55	<0.001	High
Comparison by SAA (CFU × 10^5):			
Compost of cattle manure	-34.08	<0.001	High
Compost of sapropel	-5.98	0.0103	High
Compost of SP : CM = 70 : 30	-40.06	<0.001	High

Table 5. Effect of aqueous extracts of initial components and finished compost on the characteristics of cotton seed germination

Treatment type	Cotton seed germination. pcs/day				Sprouts length		Green weight	
	3^{th} day.	±	6^{th} day	±	sm	±	g	±
Water	9.33	0.233	9.66	0.251	8.33	0.375	0.133	0.007
Sapropel 0.01%	9.67*	0.242	9.75	0.244	9.43	0.424	0.181*	0.008
Cattle manure 0.01%	9.5	0.285	9.75	0.293	10.32*	0.433	0.192*	0.008
Compost (SP : CM = 70 : 30) 0.01%	9.8*	0.412	9.925*	0.417	10.17*	0.427	0.189*	0.008
Std. Dev.	0.2044		0.1109		0.909		0.027	0.204
CI<0.05	0.3252		0.1765		1.446		0.044	
CI<0.01	0.9497		0.5155		4.224		0.128	

Note: *reliable differences from control.

difference (HSD) test (Table 4), revealed statistically significant differences between the studied samples, confirming a high level of reliability in the observed variations.

Although the compost and cattle manure samples did not show statistically significant differences in the number of heterotrophic microorganisms on meat-peptone agar (MPA), both exhibited significantly higher counts compared to sapropel. In contrast, the counts of amylolytic microorganisms on SAA differed significantly among all treatments, with the following descending order: cattle manure > compost > sapropel. The final stage of the study involved evaluating the effects of initial raw materials and finished compost on cotton seed germination and germination energy (Table 5).

This experiment was designed to assess the potential stimulatory or inhibitory impact of the materials on the early stages of plant ontogenesis, namely seed germination and initial seedling growth. It also provided insights into the phytotoxicity, maturity, and agronomic suitability of the compost as a fertilizer. To this end, aqueous extracts (0.01% concentration) of sapropel, cattle manure, and compost produced at a SP:CM ratio of 70:30 were tested. Key germination parameters—including germination rate and uniformity (on days 3 and 6), seedling length, and fresh biomass (on day 6)—were analyzed.

Previous studies have identified 0.01% aqueous suspensions of sapropel, manure, and compost as the most effective concentrations for seed treatment. Application of these extracts significantly enhanced seed germination energy, uniformity, and overall germination rate, with increases of 4.5% and 5.0% compared to the control. Additionally, a marked improvement was observed in early seedling development: average seedling length increased by 23% and 22%, while fresh biomass rose by 36%, 44.3%, and 42.1% for sapropel, manure, and compost treatments, respectively. These results indicate a statistically reliable stimulatory effect ($p < 0.05$), demonstrating the agronomic potential and low phytotoxicity of the tested materials.

CONCLUSIONS

Comprehensive chemical and physicochemical analyses of the sapropel sourced from fish farms revealed a substantial content of silicate and carbonate minerals, along with a high proportion of organic matter enriched with humic substances. To enhance the organic component and improve its fertilizing potential, co-composting with cattle manure was conducted under aerobic conditions at 25 °C for 120 days. The results demonstrated marked increases in the concentrations of humic and fulvic acids in the resulting compost.

Microbiological assessment of the compost obtained at a sapropel-to-manure ratio of 70:30 indicated the development of a specific and diverse microbial community after 90 days of maturation. This microbial profile is characteristic of a biologically active and mature organic substrate, containing compounds at various stages of humification. The findings confirm the high agrochemical and microbiological quality of the compost, supporting its potential use as an effective organic fertilizer.

Furthermore, aqueous extracts of sapropel, cattle manure, and the final compost at a concentration of 0.01% demonstrated a pronounced stimulatory effect on the early stages of cotton seed ontogenesis. A statistically significant increase in germination energy, seedling uniformity, average seedling length (by 22–23%), and fresh biomass accumulation (by 36–44.3%) was observed compared to the control. Notably, compost extracts exhibited the most pronounced positive effect, confirming the absence of phytotoxicity, the maturity of the organic product, and its suitability for use as a biofertilizer.

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